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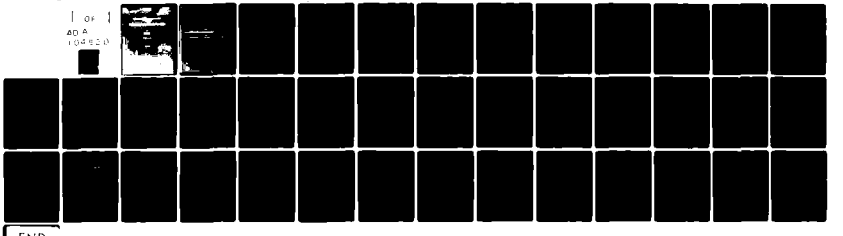

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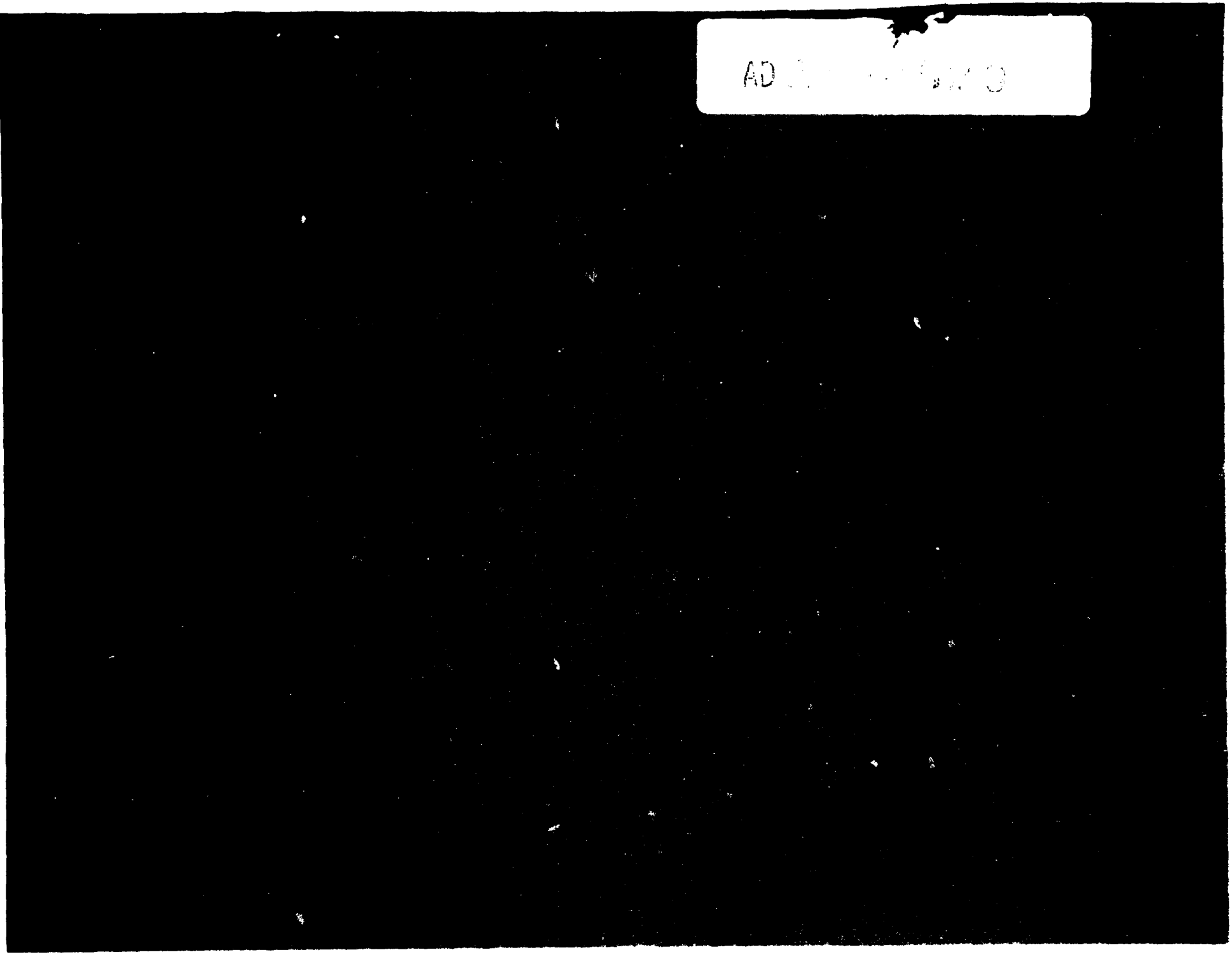

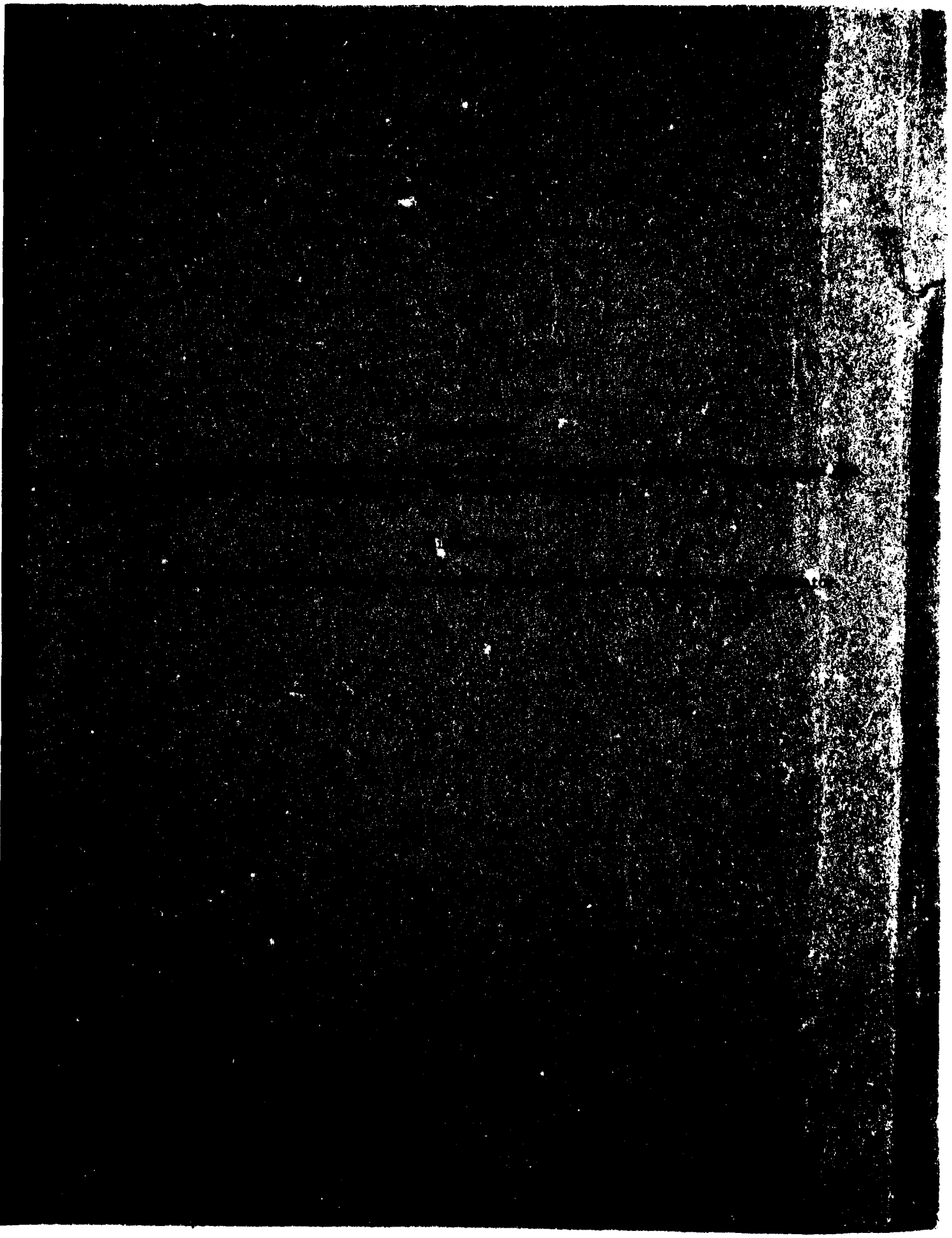

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Spectral discrimination	Tobias Acid
B-1 dye	2 Naphthylamine, 1-sulfonic acid sodium salt
p-Nitrophenylazo, 2-naphthylamine	Thin layer chromatography
Sudan Red G	

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

The objective of this work was to study the analytical chemistry of B-1 dye in the areas of process control and dye purity. B-1 dye (p-nitrophenylazo, 2-naphthylamine) exhibits a typical electronic transition in the visible portion of the spectrum. The wavelength of this transition is a function of the hydrogen bonding ability of the dye to the solvent and the polarity of the solvent. A second electronic transition for this dye occurs in the near ultraviolet portion of the spectrum and may be ascribed to the p-nitrophenyl chromophore. The ratio of absorbance of these electronic

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20. ABSTRACT (contd)

transitions, (the absorbance index) is used as a criterion of dye purity. Deviations from this ratio are shown to be related to impurities or adulterants of the B-1 dye. The newly investigated Spectral Discrimination Technique is described and found to be insensitive to impurities having similar electronic transitions to that of B-1 dye. This technique appears to have little or no value in determining B-1 dye purity.

The use of Thin Layer Chromatography (TLC) as a method of qualitatively determining the number and identity of impurities is described. TLC is a useful tool but should not be used as the sole criterion for B-1 dye purity in purchase descriptions.

On the basis of analysis of small scale production runs of B-1 dye, suggestions are made in process technique to improve the purity of the final product.

The results constituted in this report must be considered preliminary. Further investigations are warranted in the area of preparation of chromatographically pure dye by preparative liquid chromatography. This "pure" dye should be employed for the absorbance index, melting point, and decomposition temperature.

PREFACE

The objective of this work was to study various aspects of the analytical chemistry of B-1 dye in the areas of process control and end item purity.

The work described in this report was authorized under MMT Project 5781339, Manufacturing Methods and Technology for Preparation of B-1 Dye. The work was started in May 1980 and completed in September 1980. The data for this work are recorded in CSL Laboratory Notebooks 10,108 and 10,132.

The use of trade names in this report does not constitute an official endorsement or approval of the use of such commercial hardware or software. This report may not be cited for purposes of advertisement.

Acknowledgments

The authors hereby acknowledge the valuable assistance of Ms. Jennifer Hockman, Munitions Division, Laboratory Technician, for her efforts asssociated with this project.

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LIST OF SYMBOLS

λ = wavelength of radiation
λ_{max} = wavelength of maximum absorbance
Abs. = absorbance; define by the relationship: Abs = log 1/T
T = transmittance
ε = molar absorbance defined by the real relationship Abs = εbc
b = path length or thickness of solution being measured in centimeters
c = concentration of absorbing species under measurement in moles/liter
R_f = relative frontal distance defined by:

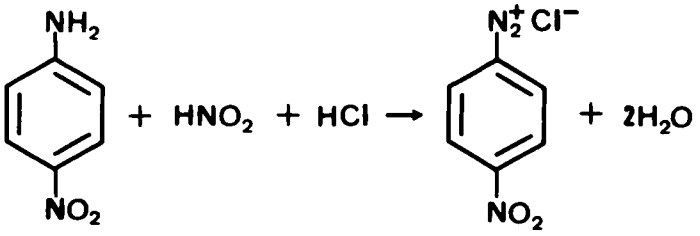
$$R_f = \frac{\text{distance of compound front}}{\text{distance of solvent front}}$$

THE ANALYSIS OF B-1 DYE (1, p-NITROPHENYLAZO 2, NAPHTHYLAMINE) BY ULTRAVIOLET-VISIBLE SPECTROSCOPY AND THIN LAYER CHROMATOGRAPHY

1. INTRODUCTION

B-1 dye (1, p-nitrophenylazo 2, naphthylamine) C. I. 11385; Solvent Red 5 is currently being employed to detect liquid chemical agent. This dye is synthesized by coupling diazotized p-nitroaniline with sodium 2, aminonaphthalene 1, sulfonate. The reaction may be depicted by:

$$NaNO_2 + HCl \xrightarrow[0^\circ \text{ to } 5^\circ C]{} HNO_2 + NaCl \qquad (1)$$



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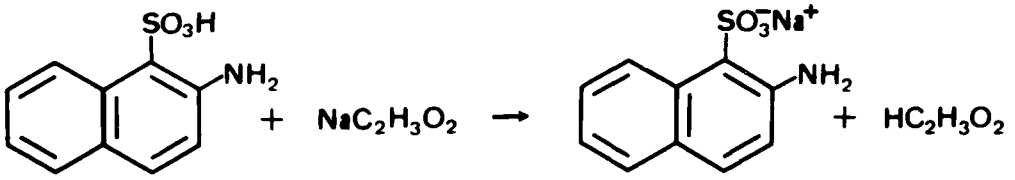

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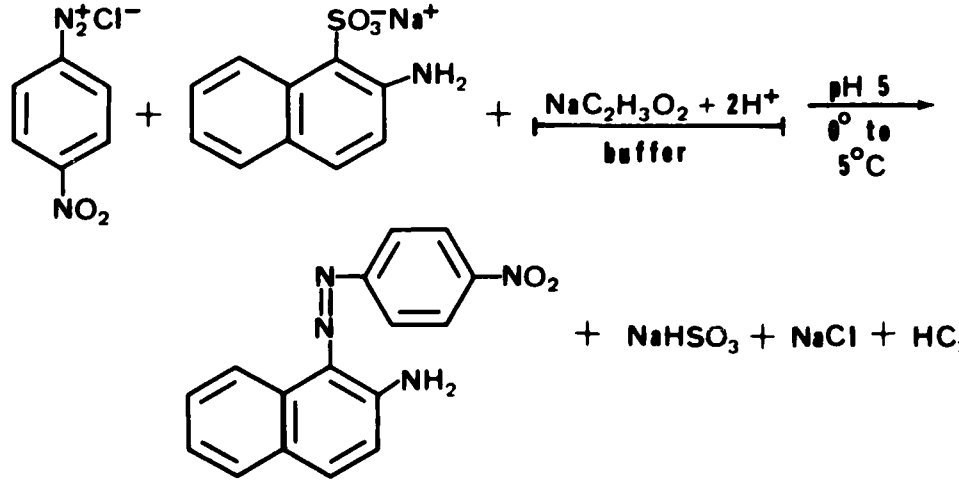

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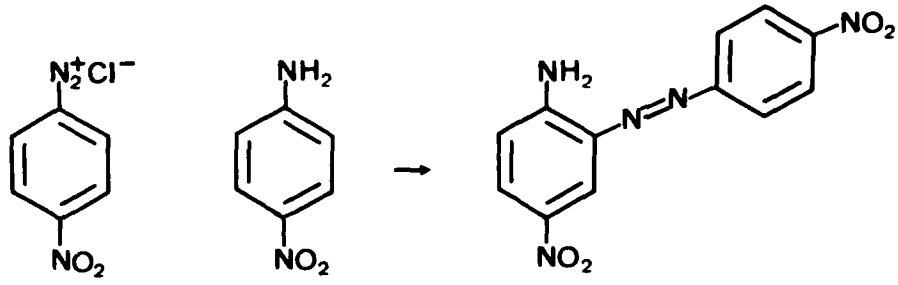
Since the dye is a derivative of β-naphthylamine, a known carcinogen, it is imperative the synthesized dye be free of this material. Sensitive chemical analysis has been devised to determine the presence of β-naphthylamine in the B-1 dye.[1] There remains the problem of determining the purity of the synthesized B-1 dye to insure product uniformity and to ascertain whether further purification is required before end use. The synthetic route to B-1 dye can lead to by-products which are potential interferents in the proposed analysis. These by-products arise from side reactions.



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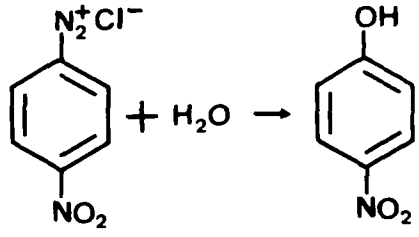

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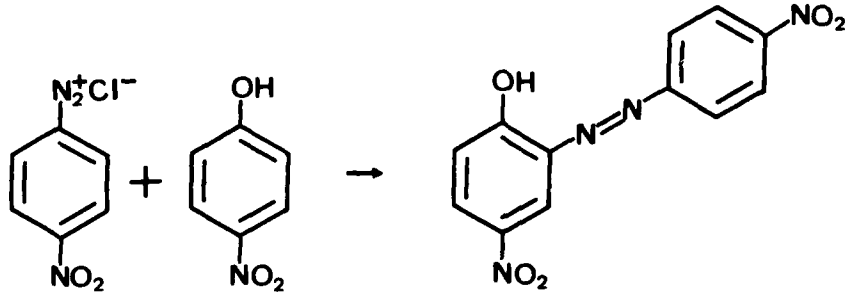

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The dye species arising by self-coupling of the diazonium salt with the unreacted precursor is shown in equation 5. Equation 6 depicts the decomposition of the diazonium salt to the corresponding phenol and its subsequent coupling to form the dye species shown in equation 7. The dye species shown in equation 7 would exhibit electronic spectra similar to B-1 dye, and analytical techniques will have to be developed to differentiate the interfering dye species from B-1 dye.

2. EXPERIMENTAL PROCEDURES

2.1 Chemicals.

2.1.1 1, p-nitrophenylazo 2, naphthylamine (B-1 dye) standard.

This standard was prepared by Federal Color Laboratories. Purity of the dye was established by dissolving 0.1 gram in 10 ml N, N dimethylformamide. A LQ-6 (Quanta/Gram^{RTM}) silica gel thin layer plate was spotted with 10 $\mu\ell$ of dye solution and the solvent allowed to evaporate. The plate was placed in a cylindrical developing chamber containing a solution of benzene and ethanol (49:1) as the ascending solvent. The solvent front was allowed to rise to a 15 cm point on the plate. The plate was removed from the developing chamber and examined while damp with solvent with long wavelength ultraviolet light. No fluorescent compounds were observed. The solvent was allowed to evaporate from the plate and only two spots were observed. The main spot, bright red, was B-1 dye and had a R_f value of 0.78. The second spot, pale violet, was barely discernible and had a R_f of 0.63. From the color intensity and size of this spot, it could not have amounted to more than 0.1% of the sample. From the chromatography data the standard dye had to be 99.9 + % pure.

2.1.2 Solvents.

(a) Methanol
(b) Cyclohexane
(c) N,N dimethylformamide
(d) Acetone
(e) Ethanol

All solvents employed in this study for spectral studies met the criteria of spectropurity.

2.1.3 All other chemicals were ACS or CP purity.

2.2 Equipment utilized.

A Beckman DK-2A Ratio Recording Spectrophotometer equipped with linear wavelength drive was utilized. Quartz spectrophotometer cells, 1.00 $\pm$ 0.01 cm path length were employed in matched pairs. Radiation sources employed for the spectrometric studies were either a tungsten lamp in the visible region or a hydrogen discharge lamp for the ultraviolet region.

All glassware used for volumetric measurement met class A, NBS, and ANBS tolerances.

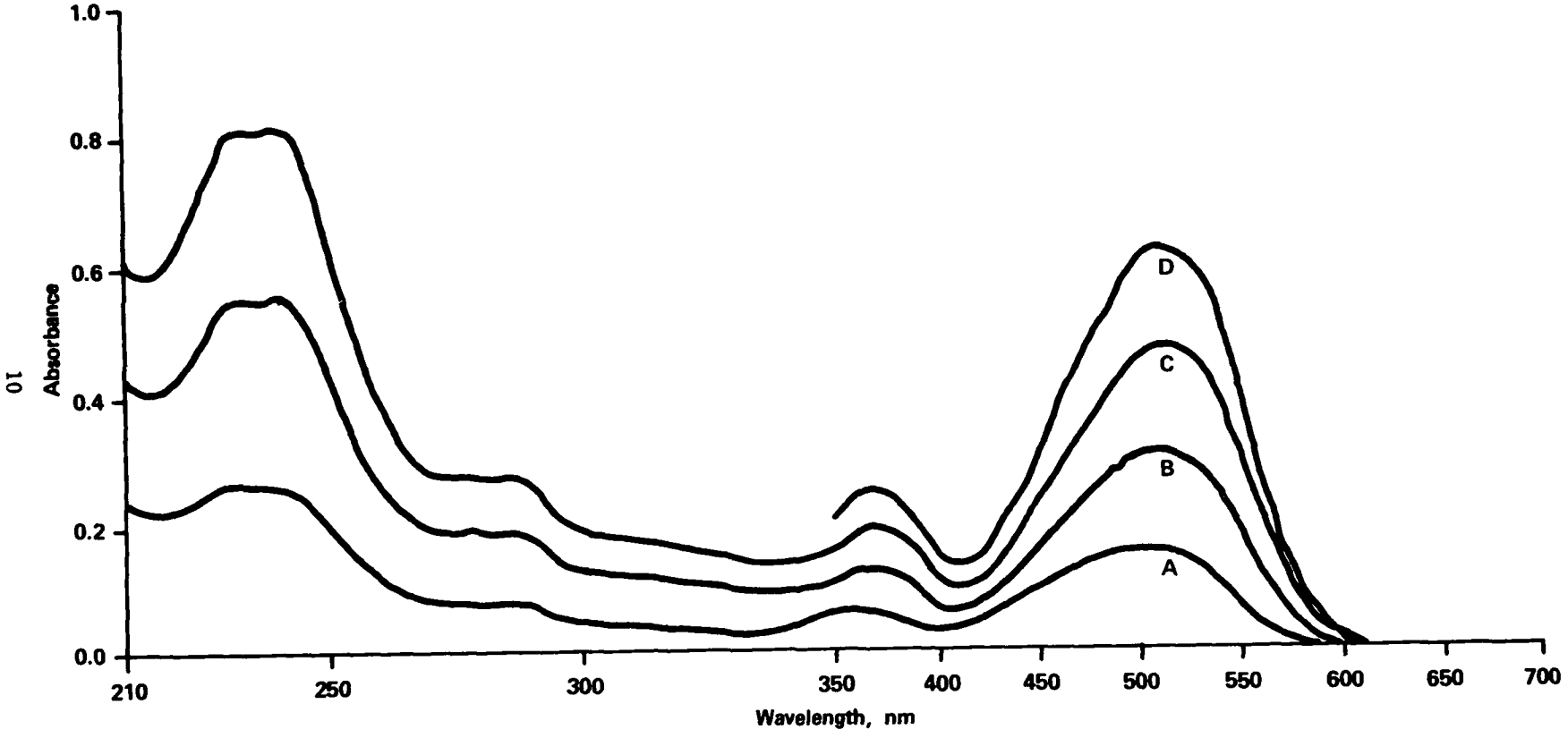


Figure 1. Spectral Characterization of B-1 Dye in Methanol

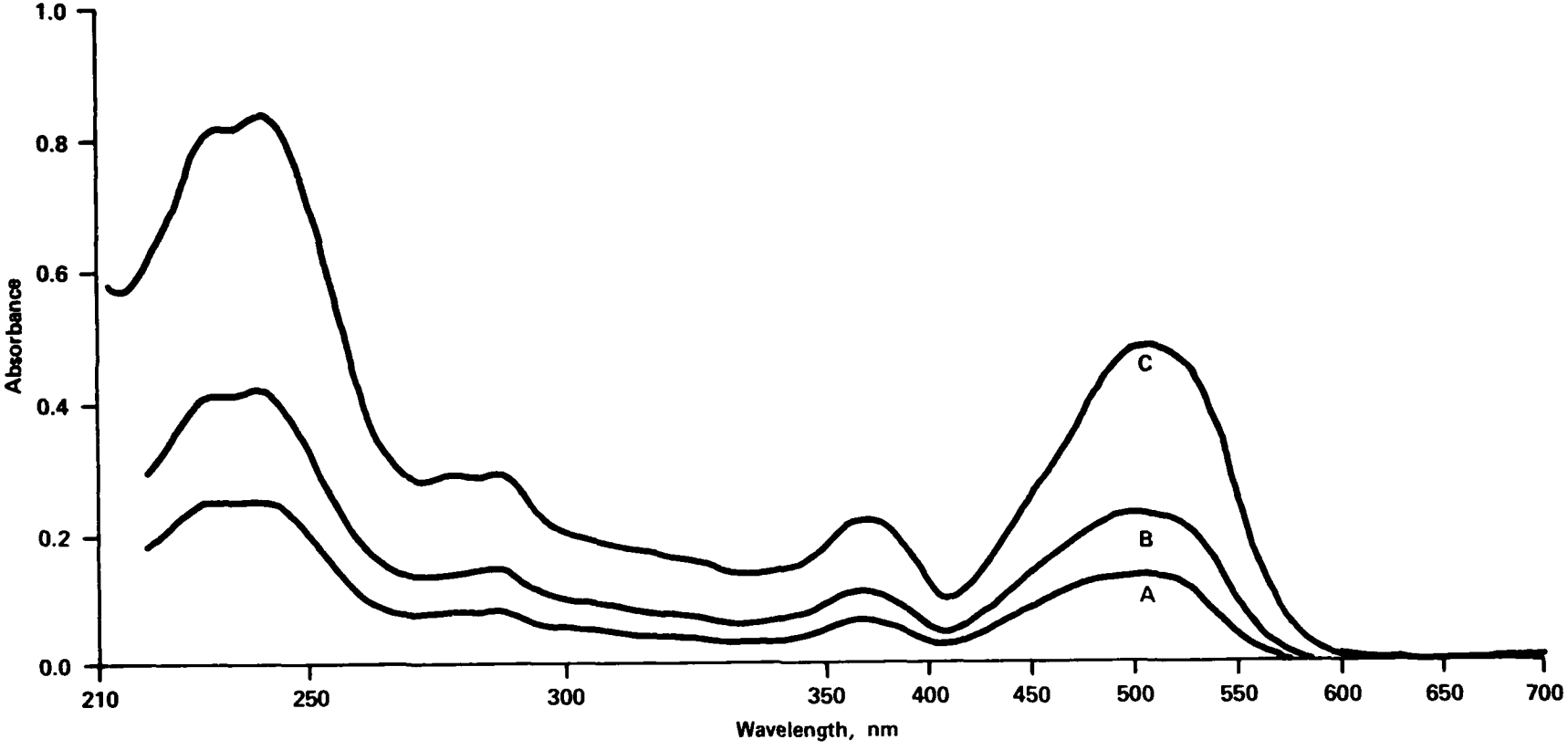


Figure 2. Spectral Characterization of B-1 Dye in Cyclohexane

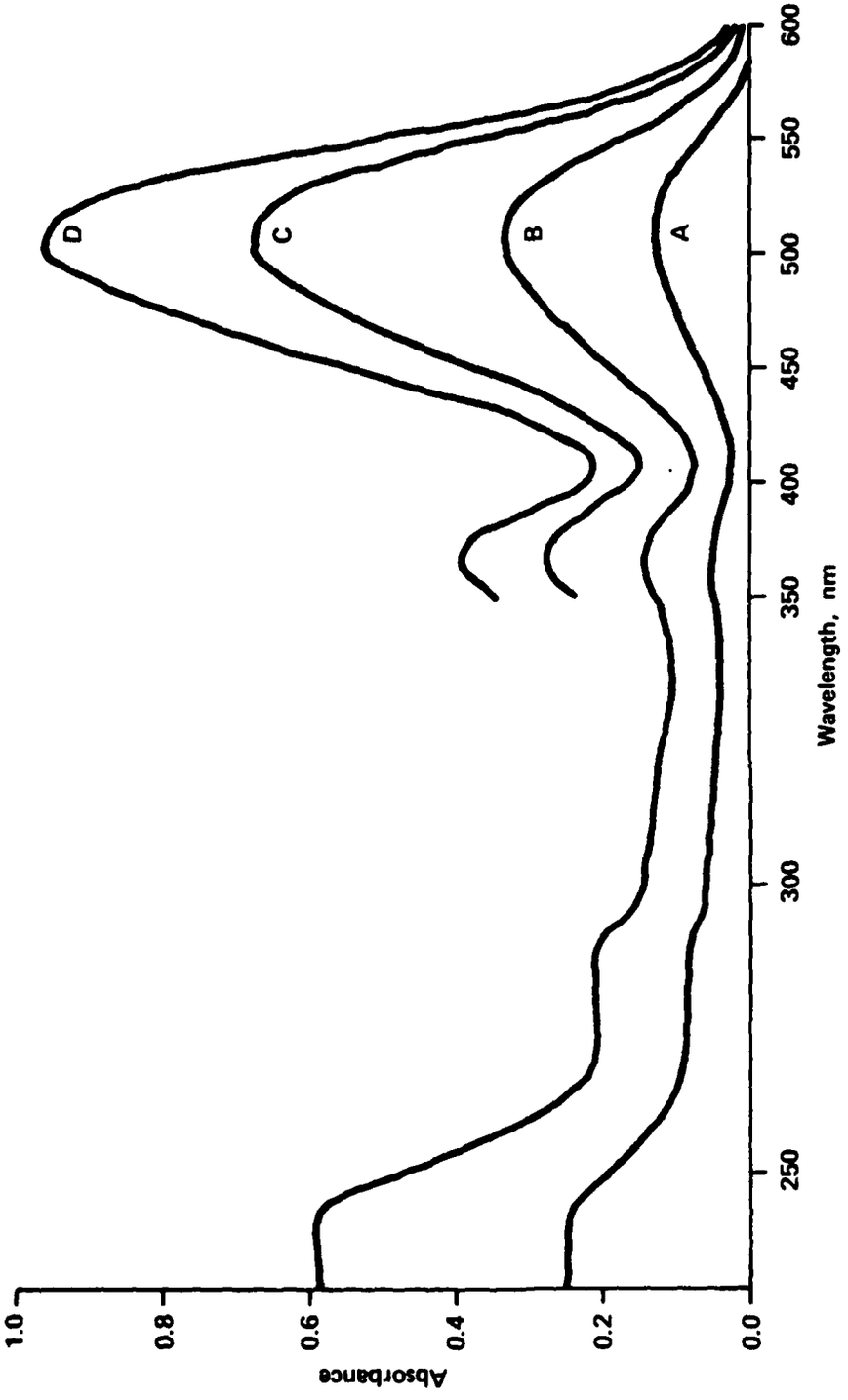


Figure 3. Spectral Characterization of P-1 Dye in Acetonitrile

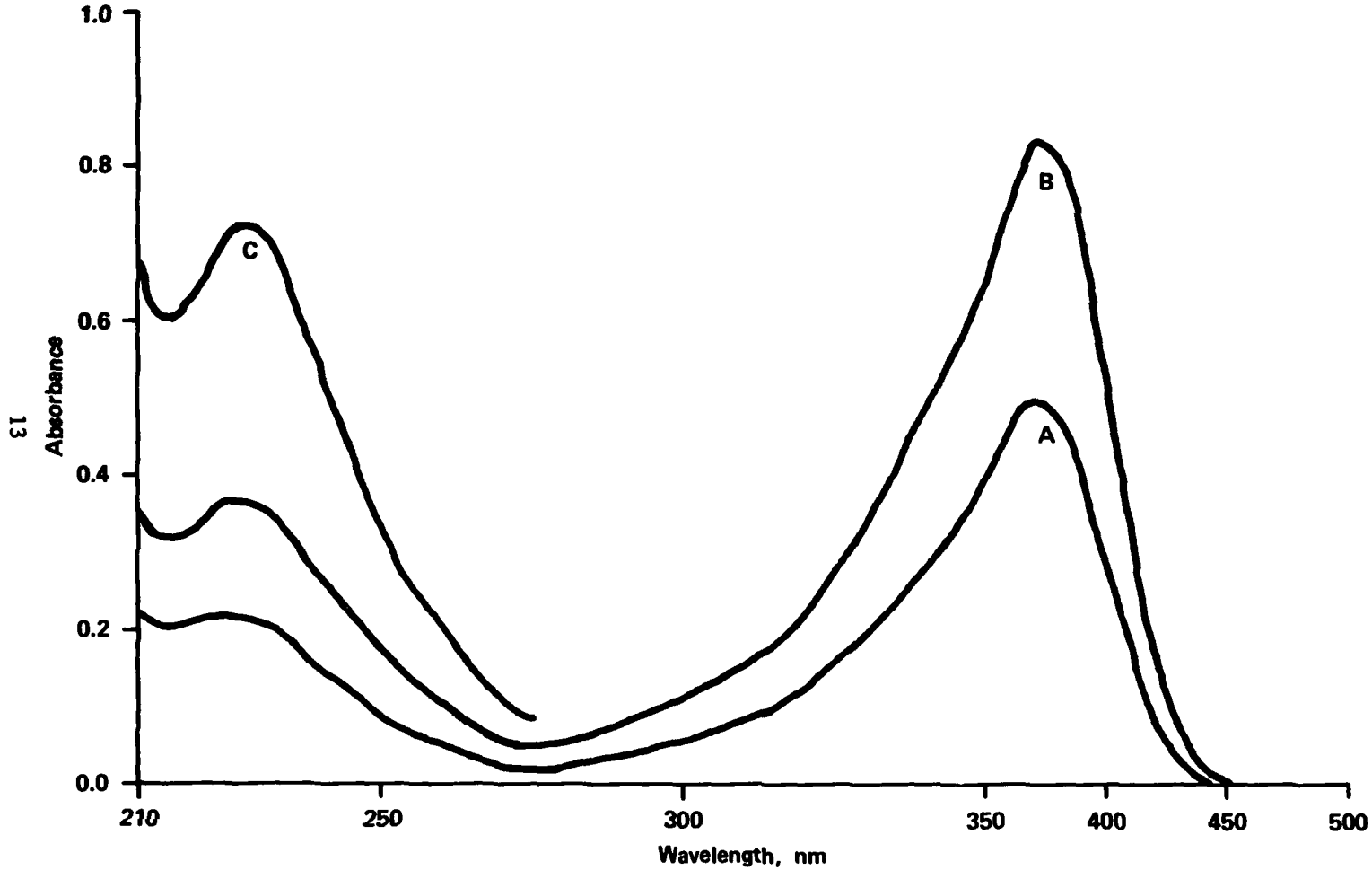


Figure 4. Spectral Characterization of p-Nitroaniline in Methanol

Table 1. The Effect of Solvent on the Electronic Spectrum of B-1 Dye

Solvent	λ_{max} nm	logϵ	λ_{max} nm	logϵ	λ_{max} nm	logϵ	λ_{max} nm	logϵ	λ_{max} nm	logϵ	λ_{max} nm	logϵ
Methanol	512.5	4.29	368	3.91	287	4.05	277	4.06	240	4.54	232	4.53
Acetone	512.5	4.29	368	3.89	*							
Cyclohexane	499	4.28	368	3.92	287	4.07	277	4.06	242	4.53	232	4.52
Acetonitrile	502.5	4.28	367	3.89	288	4.09	277	4.09	243	4.54	232	4.54

* UV cutoff for acetone is 300 nm.

Table 2. The Effect of Substitution on the Electronic Spectrum of the p-Nitrophenyl Chromophore

Compound number	X	λ_{max} nm	logϵ	λ_{max} nm	logϵ	λ_{max} nm	logϵ	λ_{max} nm	logϵ	λ_{max} nm	logϵ	λ_{max} nm	logϵ	Solvent
1	-H			260	3.91									EtoH
2	$-NO_2$			266	4.16									EtoH
3	-Cl			270.5	4.03									MeoH
4	$-CO_2H$			294	3.40									H_2O
5	-OH			310	4.00									MeoH
6	$-NH_2$			368	4.18							233	3.83	MeoH
7	B-1 dye	512.5	4.29	368	3.91	287	4.05	277	4.06	240	4.54	232	4.53	MeoH
8	$-N(CH_3)_2$			392	4.30	314	3.27					232	3.96	EtoH

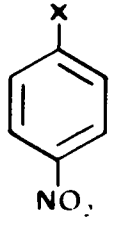


Table 3. Comparison of the Spectra of p-Nitroaniline and 2-Amino Naphthalene 1-Sulfonic Acid with B-1 Dye

Compound	λ_{max} nm	logϵ	λ_{max} nm	logϵ	λ_{max} nm	logϵ	λ_{max} nm	logϵ	λ_{max} nm	logϵ	λ_{max} nm	logϵ	λ_{max} nm	logϵ	Solvent
Tobias Acid			343	3.50	294	3.54	283	3.77	273	3.75	242	4.66	218	4.31	H_2O
p-Nitroaniline			368	4.18	294	3.54	283	3.77	273	3.75	233	3.83			MeOH
B-1 Dye	512.5	4.29	368	3.91	287	4.05	277	4.06	240	4.54	232	4.53			MeOH
Sudan Red G	512.5 *	4.33	368 *	3.64											MeOH

* Values are not λ_{max} for this dye.

Table 4. Application of the Spectral Discrimination Technique to B-1 Dye

Sample	Absorbance at wavelength			
	475 nm	512 nm	525 nm	537.5 nm
9.32 $\frac{mmg}{ml}$ Pure B-1 dye	0.477	0.619	0.600	0.526
Mixture: 8.61 $\frac{mmg}{ml}$ B-1 dye + 1.11 $\frac{mmg}{ml}$ Sudan Red G	0.500	0.641	0.610	0.520

3. PREPARATION OF DYE SOLUTIONS FOR SPECTRAL CHARACTERIZATION

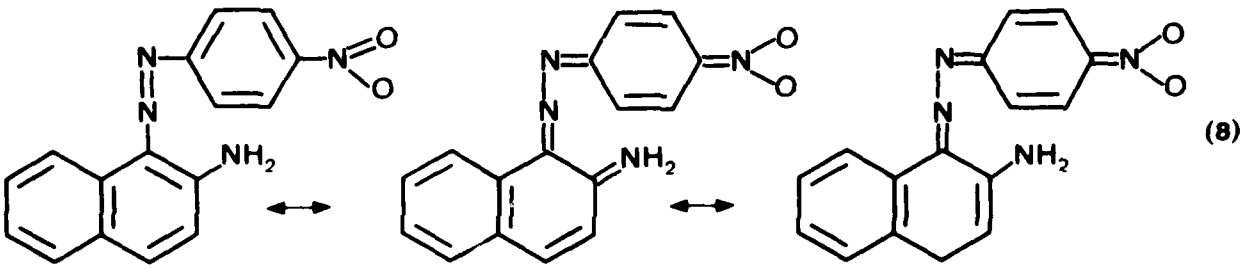
Approximately 0.1 gram $\pm$ 0.1 mg was weighed into a previously tared volumetric flask and brought to volume with solvent. The dye was brought into solution by shaking or placing in an ultrasonic bath. Subsequent dilution to the concentration range suitable for spectrophotometric characterization was accomplished by standard volumetric technique. Cyclohexane is a non-polar aprotic solvent and is useful in studying the effect of intra-hydrogen banding in the dye molecule. Since B-1 dye is not soluble in cyclohexane, the following technique was employed to characterize the dye in this solvent. The dye sample was first dissolved in a minimal quantity of methanol. The flask was then brought to volume with cyclohexane using drop wise addition of methanol to keep the dye in solution. All subsequent volumetric dilutions of the initial standard solution were made with cyclohexane. The solutions that were spectrophotometrically characterized were below 1% in methanol by volume.

4. RESULTS AND DISCUSSION

4.1 Ultraviolet-visible spectroscopy.

The electronic spectrum of a methanolic solution of B-1 dye is shown in figure 1. The intense band in the 500 nm region is due to the interaction of the basic chromophore, the azo group, with the substituents. The influence of the auxichrome, the 2-amino substituent on the naphthalene ring and its interaction with solvent is manifest by the position of λ_{max} in the visible region by B-1 dye in cyclohexane, and acetonitrile, (figures 2 and 3). Table 1 shows that B-1 dye exhibits a bathochromic shift in polar protic solvents such as methanol and acetone. This shift to a longer wavelength is due to hydrogen bonding between the dye and solvent, resulting in effectively lowering the energy of the electronic transition when compared to the position in a nonpolar, aprotic solvent such as cyclohexane. The intensity of the transition, noted by logϵ is not affected by the polarity of the solvent.

The primary chromophore, the azo group, has a λ_{max} at 400 nm when substituted by aromatic substituents.[2] The bathochromic shift to the 500 nm region is due to the electron withdrawing property of the nitro group in the 2 position of the naphthalene ring. The color observed for the dye is due to a $n \rightarrow \pi^*$ electronic transition. This effect can be depicted by:



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Though the p-nitrophenyl group is a substituent on the 1-naphthylazo chromophore group, it is a chomophore in its own right. The electronic spectrum of a methanolic solution of p-nitroaniline is shown in figure 4. Table 2 lists the position of λ_{max} and log ϵ of the electronic transitions of a selected number of p-nitrophenyl compounds. In nitrobenzene, (compound 1), the λ_{max} of the lowest energy electronic transition is 260 nm. In p-nitroaniline, (compound 3), the lowest energy electronic transition is 368 nm. Pure B-1 dye, (compound 7), exhibits this identical electronic transition at 368 nm. Thus, the absorbance at this wavelength can be utilized as an additional point of reference to establish dye purity. The influence of electron releasing substituents in the 4 position in lowering the energy of the first electronic transition is clearly shown in table 2 with compounds 5, 6, and 8, while the influence of electron withdrawing substitution is indicated with compounds 2, 3, and 4.

The measurement of the absorbance of B-1 dye at 512.5 nm is due to a $n \rightarrow \pi^*$ electronic transition of the azo group. Similar $n \rightarrow \pi^*$ transitions occurring in the various dyes formed by side reactions during synthesis will have similar spectral characteristics (see Introduction). Measurement of absorbance at only one wavelength, 512.5 nm, can result in erroneous values of B-1 dye purity.

Shown in table 3 are the values of the absorbance of B-1 dye at 512.5 nm and 368 nm.

The value of $\frac{\text{Absorbance 512.5 nm}}{\text{Absorbance 368 nm}} = R$, where R = absorbance index. (9)

The value can be obtained from $\log \epsilon_{512.5\ \text{nm}} - \log \epsilon_{368\ \text{nm}} = \log R$ $\log R = 4.29 - 3.91 = 0.38$, $R = 2.40$.

This ratio determined for "standard" pure B-1 dye can be employed to determine the purity of commercially produced B-1 dye. The electronic spectrum of aqueous 2-aminonaphthalene-1-sulfonic acid (Tobias Acid) is shown in figure 5. The wavelength of the absorbance maximum, λ_{max} and $\log \epsilon$ values for Tobias Acid is shown in table 3. Also included in this table are the values for B-1 dye and p-nitroaniline.

1-(2-methoxyphenyl) azo 2-naphthol (Sudan Red G) is an azo dye that has a structure similar to B-1 dye. The electonic spectrum of Sudan Red G in methanol is shown in figure 6 and in acetonitrile in figure 7. Table 3 lists the λ_{max} and $\log \epsilon$ values for this dye in the visible and ultraviolet regions of the spectrum. Figure 8 is the spectrum for B-1 dye containing 11% by weight Sudan Red G in methanol. Comparison of this spectrum in the visible region with that of the pure dye shown in figure 1 indicates little change in the general shape of the spectrum. Measurement of the absorbance of the dye mixture at λ_{max} of 512 nm yielded a value equivalent to 97.0% B-1 dye. Clearly measurement at a single wavelength of λ_{max} in the visible region does not discriminate between these two dyes and, in this instance, resulted in a positive error of 8.4%.

The Spectral Discrimination Technique developed by Perkin Elmer Corporation was applied to these spectra.[3] Shown in table 4 are the results of comparison of the spectra of pure B-1 dye with that of the mixture of 88.6% B-1 dye and 11.4% Sudan Red G. Comparison of the absorbances at each wavelength yields a q value defined by:

$$q = \frac{\text{Abs. of pure B-1 dye at } \lambda_1}{\text{Abs. of dye mixture at } \lambda_1} \quad (10)$$

$$q_{475\ \text{nm}} = 0.954$$
$$q_{512\ \text{nm}} = 0.966$$
$$q_{525\ \text{nm}} = 0.983$$
$$q_{537.5\ \text{nm}} = 1.01$$

The ratio of q values at various wavelengths yields the value D, the Spectral Discriminator, as defined by:

$$D = \frac{q\,\lambda_1}{q\,\lambda_2} \quad (11)$$

$$D = \frac{q_{512\ \text{nm}}}{q_{475\ \text{nm}}} = \frac{0.966}{0.954} = 1.01$$

$$D = \frac{q_{525\ \text{nm}}}{q_{512\ \text{nm}}} = \frac{0.983}{0.966} = 1.02$$

$$D = \frac{q_{537.5\ nm}}{q_{512\ nm}} = \frac{1.01}{0.966} = 1.05$$

$$D = \frac{q_{537.5\ nm}}{q_{525\ nm}} = \frac{1.01}{0.983} = 1.03$$

$$D = \frac{q_{537.5\ nm}}{q_{475\ nm}} = \frac{1.01}{0.954} = 1.06$$

average $D = 1.03 \pm 0.02$.

According to Paile et al.,[3] D values generally fall between values of 1 to 1.5 and that values greater than 1.5 indicate dissimilar compounds. The value of 1.03 ± 0.02 indicates the two spectra are virtually identical. Thus, the spectral discrimination technique failed to note any differences in the spectral shape in the visible region. One could conclude using this technique that the mixture was indeed pure B-1 dye.

The method of Absorbance Index was applied to the Sudan Red G Dye mixture as shown in table 5. From these data the ratio of Absorbance Index of the dye mixture to the pure B-1 dye was calculated.

$$R^* = \frac{R_{mix}}{R_{pure}} \times 100\% = \frac{2.68}{2.40} \times 100 = 111.7\%$$

The high value of R for the dye mixture indicates the presence of 11.7% of an absorbing dye species (other than B-1 dye) at 512 nm.

4.2 Thin layer chromatography (TLC).

TLC is a technique used to separate compounds in a mixture rapidly and, in most instances, the separation is quantitative. The technique has a deficiency in that only small amounts of materials can be separated and quantitation of the individual compounds is difficult. Quantitation is generally performed by either of two techniques; measurement of reflectance with a spectroreflectometer or scraping the solid containing the spot of interest, or extraction of the compound off the solid support with a suitable solvent followed by a spectrophotometric finish. Both techniques have inaccuracies.

Spectroreflectance measurements depend on the surface concentration of the reflecting species. Therefore, high concentrations of reflecting species concentrated to a small area (such as B-1 dye which would constitute 95% to 99% by weight of the applied samples) would result in negative errors. This is due to successive layers of dye being deposited upon one another which results in masking the dye from the reflectance measurement. Measurement of the concentration of impurities would require prior knowledge as to the identity of each impurity, so that calibration curves can be prepared. Since there is a possibility for a plethora of impurities resulting from impurities in starting materials and side reactions occurring during diazotization and coupling, it therefore appears that quantitative determination of impurity level would be an extremely complex task.

Scraping of the B-1 dye spot from the TLC plate can be performed, but the problem of quantitative removal of the dye from the solid absorbent with solvent can prove difficult. This technique can lead to negative errors due to incomplete desorbtion of dye from the silica gel absorbent. In addition, relatively small quantities of sample, generally less than 1 mg, can be successfully chromatographed. The sample has to be spotted on the plate in a small volume, therefore dye solutions of high concentration must be utilized in spotting so that sufficient

separated material is present in the solvent extract for accurate spectrophotometric measurement. Hence, small errors in application of test solution volume will result in relatively large errors in recovered B-1 dye concentrations. In view of these considerations, the application of TLC as a quantitative technique to determine B-1 dye purity is of doubtful utility. However, the ability of TLC for facile separation of B-1 dye from impurities should not be overlooked as an important analytical technique to qualitate the presence of dye impurities.

To illustrate the utility of TLC to yield information of use in the B-1 dye problem, one can point to a series of B-1 dye samples submitted by CSL's CB Detection and Alarms Division, Detection and Alarms Producibility and Engineering Branch. These samples are the result of a small scale pilot synthesis and were analyzed by ultraviolet-visible spectroscopy and TLC. The technique employed is illustrated in the flow chart shown in figure 9. A summary of the analysis results is presented in table 6. Thin layer chromatography was performed by the method described in the experimental section in determining the purity of "standard" B-1 dye. The R_f values for the dye and its progenitors are shown in table 7.

One notes excellent separation of B-1 dye from the starting materials. However, in actual chromatograms of synthesized B-1 dye numerous other compounds were separated. Many of these compounds were highly fluorescent under long wavelength ultraviolet light. It is conjectured that these compounds arise from impurities in the Tobias Acid or result from side reactions occurring during the diazotization and coupling reactions. Noted also in these samples were numerous colored impurities. The R_f values for these impurities are shown in table 8.

4.3 Application of these studies to B-1 dye production and procurement.

Extensive discussions with personnel involved in the B-1 dye produced from small scale pilot synthesis have revealed a number of pitfalls to be avoided should actual large scale production become necessary. The following suggestions are apropos on the basis of the B-1 dye samples analyzed by the techniques described. Many of these suggestions are well documented in the open literature describing synthesis of azo dyes and are directly applicable to B-1 dye synthesis.

- The starting materials for the B-1 dye synthesis, p-nitroaniline and Tobias Acid should be of highest purity available. This will insure a product with a minimal quantity of extraneous by-products.

- The p-nitroaniline should be completely dissolved in the aqueous media prior to diazotization. Because of the decreased basicity of p-nitroaniline at least a three-fold excess of hydrochloric acid above the calculated stoichiometric amount should be employed. The resulting solution should then be cooled to about 4°C and filtered into the reactor.

- During diazotization, the temperature should be carefully monitored. Ice should be added to the reaction mixture to insure the temperature never exceeds 4°C. Good stirring is prerequisite to successful diazotization, as is slow addition of the nitrite solution. Upon complete addition of the nitrite solution, a test for the presence of free nitrous acid should be performed with starch iodide solution or paper. The excess nitrous acid should be destroyed with the addition of a cold solution of urea. Large excesses of urea should be avoided.

- The Tobias Acid should be dissolved in an aqueous acetate buffer of sufficient strength such that upon addition of the diazo solution, the final pH will be about 5.

- The buffered Tobias Acid solution should be cooled to about 10°C before the diazo solution is added to it with rapid stirring. The temperature of the mixture should be allowed to rise slowly to ambient.

Table 5. Application of the Absorbance Index Technique to the Determination of B-1 Dye Purity

Pure B-1 dye concentration mmg/ml in methanol	Absorbance at 512 nm	Absorbance at 368 nm	$R = \frac{\text{Abs 512 nm}}{\text{Abs 368 nm}}$
3.30	0.208	0.078	2.67
6.60	0.443	0.187	2.37
13.20	0.880	0.254	2.49
19.80	1.332	0.544	2.45
			Average R = 2.50 $\pm$ 0.11
Mixture 88.6% pure B-1 dye 11.4% Sudan Red G mmg total dye/ml in MeOH			
14.58	0.950	0.352	2.70
9.71	0.642	0.242	2.65
7.28	0.479	0.179	2.68
			Average R = 2.68 $\pm$ 0.02

Table 6. Results of Analysis of Crude B-1 Dye

Sample number	% Acetone insoluble	% Acetone soluble	% B-1 dye present in acetone soluble material	R_{obs}	% B-1 dye purity	% B-1 dye in sample
1 - Run 9 1/2 hour coupling	47.6	52.3	Not detectable[a]			
2 - Run 9 1 hour coupling	37.7	62.3	Not detectable[a]			
3 - Run 9 1 1/2 hours coupling	47.1	52.9	48.1[b]	1.31	54.6[c]	25.4
4 - Run 9 2 hours coupling	46.7	53.3	58.5[b]	1.38	57.5[c]	31.1
5 - Run 9 2 1/2 hours coupling	49.9	50.1	61.6[b]	1.57	65.4[c]	30.7
6 - Run 9 2 1/2 hours coupling washed crude 2 hours	0.3	99.7	101.3[b]	2.51	104.6[c]	101.0

[a] TLC indicate at R_f for B-1 dye no red spot visible to naked eye. Sample was non-homogeneous.

[b] Calculated from absorbance at 512 nm.

[c] Calculated from % Dye Purity = $\frac{R_{obs}}{R_{PURE\ B\text{-}1\ dye}} \times 100$.

Table 7. R_f Values for B-1 Dye and Impurities Obtained by Thin Layer Chromatography*

Compound	R_f
B-1 dye (1-nitrophenylazo 2-naphthylamine)	0.78
p-nitroaniline	0.53
Tobias Acid (2-amino naphthalene 1-sulfonic acid)	0.03**

* Solvent - Benzene/Ethanol (49:1) Plate - Quanta/Gram$_{TM}$ LQ6-D ascending solvent front allowed to rise 15 CM.

** Vivid blue fluorescence under long wavelength ultraviolet light.

Table 8. R_f Values of Impurities Appearing in Production Lot of B-1 Dye

R_f	Color of Spot	Compound
0.53	Yellow - brown	(p-nitroaniline)
0.54	Orange - red	
0.55	Yellow orange	
0.78	Red	(B-1 dye)
0.83	Light orange	
0.86	Light violet	
0.90	Light pink	
0.92	Faint yellow	

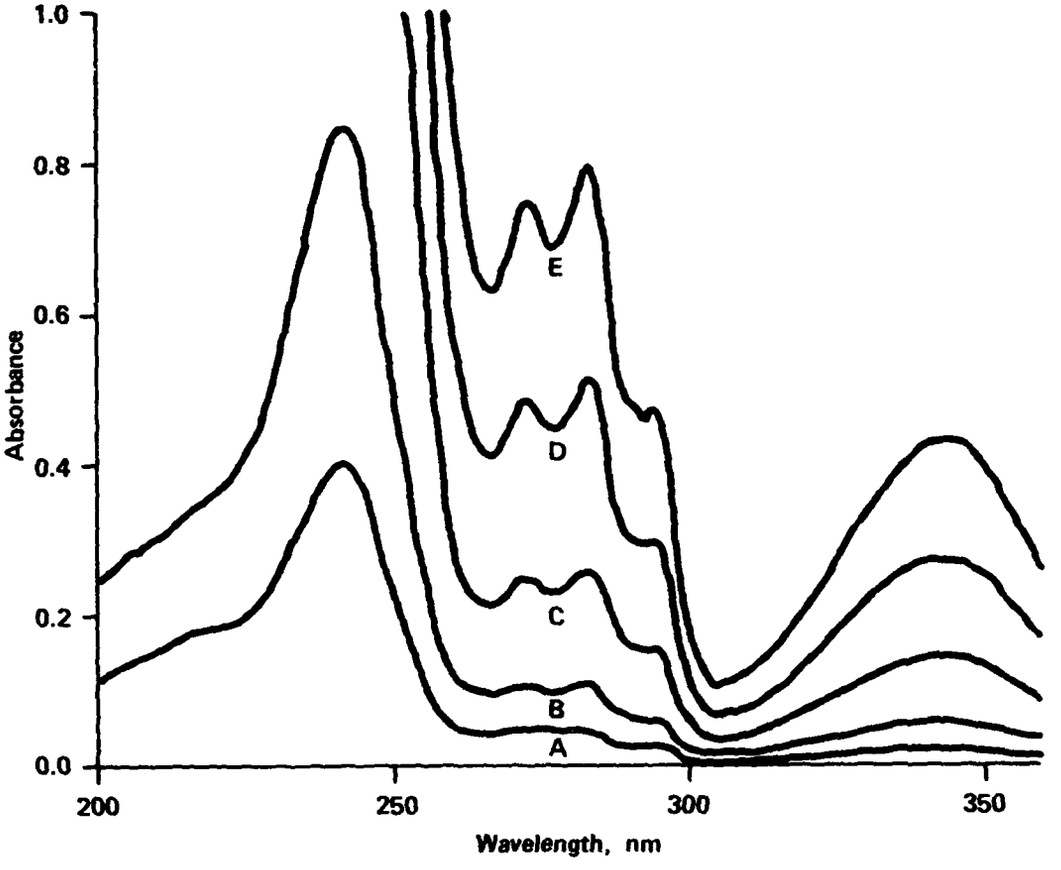

Figure 5. Spectral Characterization of Tobias Acid in Water

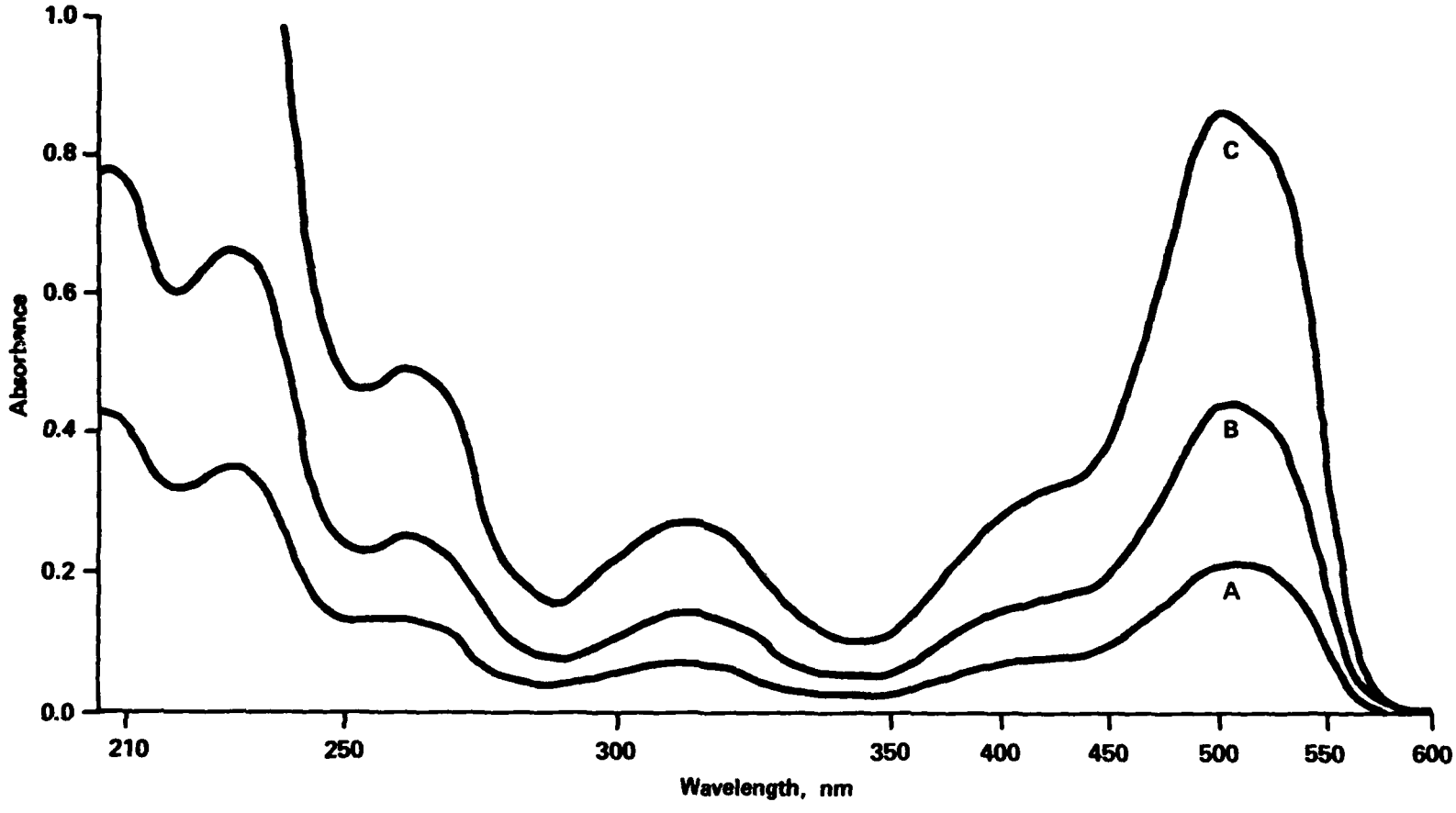

Figure 6. Spectral Characterization of Sudan Red G in Methanol

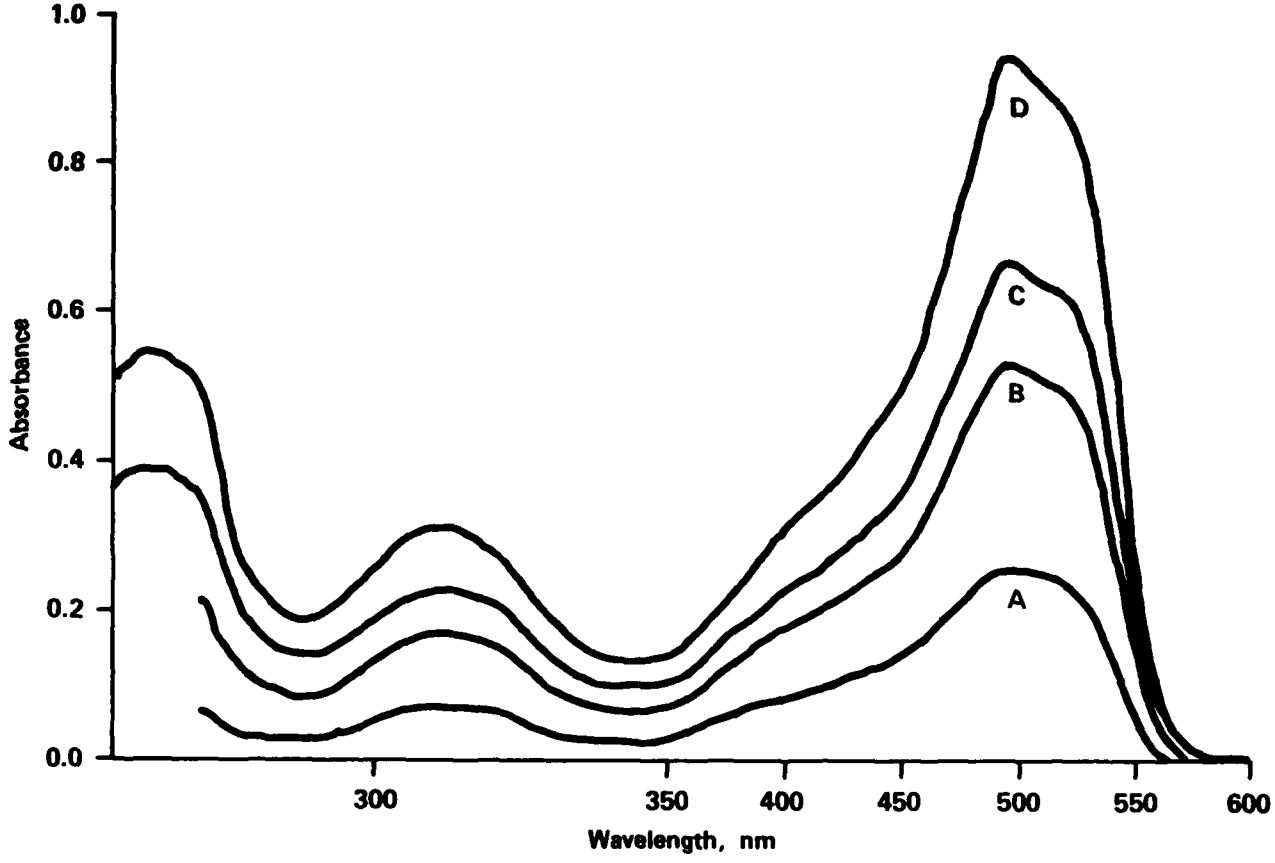

Figure 7. Spectral Characterization of Sudan Red G in Acetonitrile

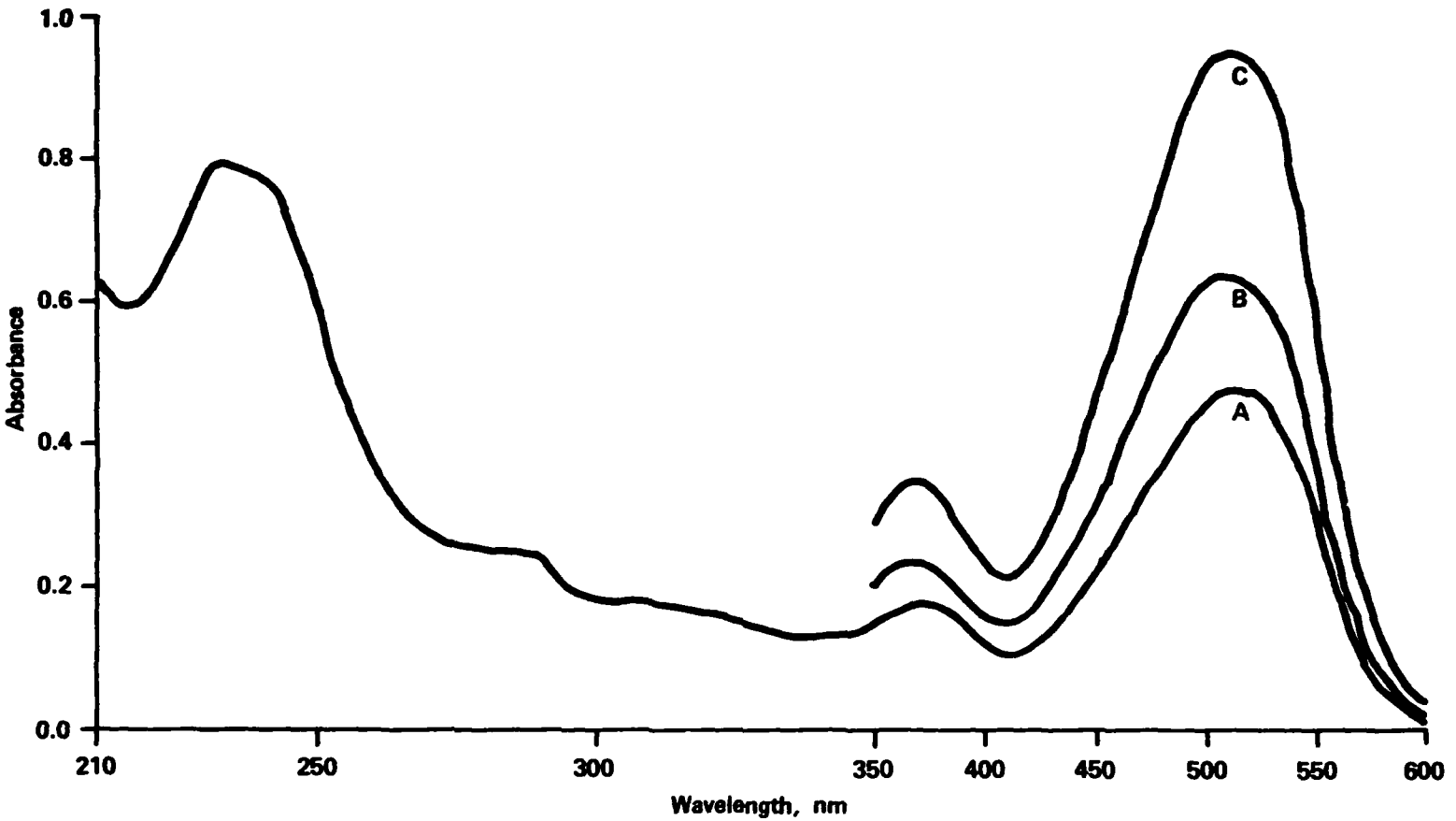

Figure 8. Electronic Spectrum of B-1 Dye Containing 11% by Weight Sudan Red G in Methanol

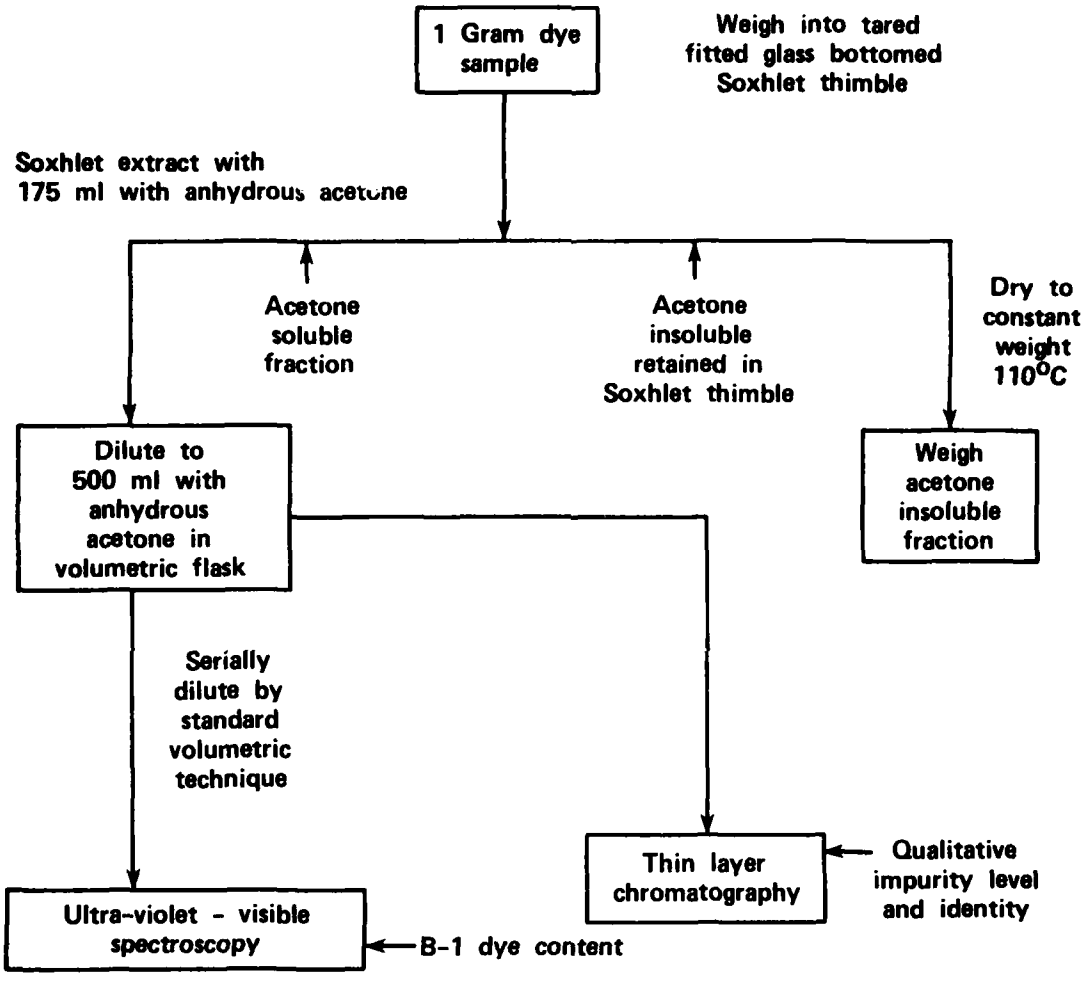

Figure 9. Analysis Scheme for B-1 Dye Produced by Pilot Scale Synthesis

• The crude B-1 dye should be filtered by suction through a strong retentive filter media such as polyester or dacron cloth. Good washing is a critical operation. Initial washing with water at 70°C with frequent stirring of the precipitate is required. Final washing should continue with water at ambient temperature until the washings are colorless and free of acid. The pH of the final washings should be that of the wash water.

• The water in the B-1 dye contained in the suction filter should be drawn off until a very slightly damp filter cake is formed. The damp cake is then transferred to glass trays, and the cloth filter media is removed. The dye can be dried in a vacuum oven at 50°C or in a steam or electrically heated convection draft oven at 70°C. Care should be taken that the dye is not placed over a heating element in an electrically-heated oven; hot spots are to be avoided. The ultraviolet-visible spectrum from 700 nm to 220 nm using pure methanol as a solvent should be obtained from a weighed sample of the dried dye. The absorbance at 512.5 nm and 368 nm should be measured and an R value calculated. The overall spectrum should be carefully compared to that of "pure" B-1 dye for extraneous absorption bands.

The current draft purchase description for B-1 dye is inadequate since it does not include a minimal purity value for the dye. Rather, the purchase description relies on a maximal allowable value for free B-naphthylamine. TLC is employed to determine the presence of impurities. The infrared spectrum of the purchased dye is used to establish material identity by listing the wavelength of the main absorbing bands with only a qualitative description of the oscillator strength of these bands. Production personnel are concerned with the actual performance of the B-1 dye in end item usage. The relationship of dye purity and end item function has yet to be examined. To this end the current draft purchase description requires particle size values of the procured dye to fall within a prescribed range. However, the purchase description should include the criteria for production acceptability of B-1 dye lots for end item usage. This would allow the rejection of dye lots that fail to function correctly when formulated into the end item.

The draft purchase description indicates that the government will furnish contractors "standard" B-1 dye as criteria of purity. Definition as to what constitutes "pure" B-1 dye has yet to be determined. The furnishing of a standard "pure" sample to a contractor without some means of packaging to preserve chemical purity by protecting against moisture and photo decomposition are areas deserving of some consideration. It has been observed that contractors in possession of other government furnished "standard purity" dye samples, were given no instructions as to the conditions of storage. "Standard" dyes were stored in clear glass vials kept in desk drawers, and, in one instance, among other laboratory organic chemicals. Standard B-1 dye samples should be furnished in 1 gram quantities. The dye should be in clean, dryscrew-capped vials. The cap should have a teflon liner. The vial should be made opaque by covering with aluminum foil, held in place with masking tape. The cap should be sealed to the vial with Pliofilm™ to act as a vapor barrier. The sample should be placed in a vapor seal bag containing a dessicant. Detailed directions as to storage of the sample after the vapor seal bag is broken should be given.

It would be desirable that the purchase description for B-1 dye state a minimal value of dye purity. This is necessary because the dye will be purchased on a weight basis, and the information of dye purity can be an important criterion in determining dye lot acceptability. Purity is currently being performed by comparison to a government furnished standard by measurement of absorbance of equal weights of dye standard and dye sample in alcohol solution at 510 nm. Though this procedure functions well as shown by the results obtained with the process samples analyzed that contained only B-1 dye and unreacted p-nitroaniline, it is also capable of giving false answers. This is shown by the results obtained in the analysis of the B-1 dye/Sudan Red G dye mixture. It should be pointed out that an infrared spectrum of such a mixture would show B-1 dye spectrum distinctly (figure 10). It has been shown that measurement of the

absorbance of B-1 dye at two wavelengths, (368 nm and 512.5 nm), yields a ratio that is invariant for B-1 dye. The general shape of the spectrum can be compared to pure B-1 dye to insure that the position of the λ_{max} in the visible region falls within stated limits for the submitted samples. Thus, the qualitative morphological aspects of the absorption spectrum of the B-1 dye/Sudan Red G mixture is that of B-1 dye; the ratio of absorbance at 368 nm and 512.5 nm clearly indicates a divergence from that of pure B-1 dye. An unscrupulous supplier of B-1 dye could upgrade a poor B-1 dye lot by blending in another dye having an absorbance at 512 nm and meet requirements for acceptability. However, this type of fraud would be evident by the two wavelength measurement method. One could argue that TLC would separate out the adulterant from the B-1 dye. One must point out that the R_f value for pure B-1 dye is 0.78 and that for Sudan Red G is 0.65, using the TLC plates and solvent system specified in the purchase description. Since both dyes in this example are red and the separation by TLC is marginally good, convincing argument could be made that the purchase description is incorrect and that the adulterated dye was B-1 dye on the basis of infrared and visible spectrophotometry. This sort of situation could lead to production of poor end items, or litigation and delays in end item production. It is believed that the scheme of measurement of B-1 dye purity at two wavelengths offers a way of avoiding this sort of situation and insuring that purchased dye lots are B-1 dye. In addition, the value of the ratio of absorbance at 368 nm and 512.5 nm indicates the nature of the impurity.

In the studies conducted in this laboratory the principal impurity detected was p-nitroaniline. This impurity resulted from incomplete diazotization and incomplete washing of the product. Since p-nitroaniline absorbs ultraviolet radiation at 368 nm, the value of R in equation 9 decreases, and the extent of decrease is proportional to the concentration of this impurity. Figure 11 shows the electronic spectrum of p-nitrophenol. This compound results from the hydrolysis of p-nitrophenyldiazonium chloride when temperatures exceed 10°C. Though λ_{max} for p-nitrophenol occurs at 317 nm, the absorption band is sufficiently broad such that the $\log\epsilon$ at 368 nm is 3.3. Therefore, should partial hydrolysis of the p-nitrophenyldiazonium chloride to p-nitrophenol occur, its presence would be indicated by a low value of R. Adulteration of the B-1 dye with an absorbing species in the 512 nm range would result in a high value of R. The characterization of B-1 dye purity depends on what is considered "pure." The current "pure" material is quite good, but still has distinct traces of impurities in it. For purposes of purchase descriptions and assaying purity of material produced during synthesis, this "pure" dye is adequate. However, for the purposes of establishing physiochemical measurements, such as molar absorbance values, the value of R, etc., a purer sample would be desirable. Due to time and budgeting limitations of this present study, a "chromatographically pure" B-1 dye sample could not be prepared. It is suggested that a process liquid chromatograph be employed to prepare a quantity of "chromatographically pure" B-1 dye. This sample should be carefully preserved and used for physiochemical measurements and referee purposes. Our studies in purifying B-1 dye by fractional crystallization were only marginally successful. High purity dye could not be produced by repeated crystallization from various solvents such as glacial acetic acid, methyl cellosolve, methyl ethyl ketone, and ethanol. It appears that the only viable method for producing the high purity B-1 dye required for physiochemical measurement is liquid chromatography.

5. CONCLUSIONS

The results constituted in this report must be considered preliminary. Further investigations are warranted in the area of preparation of "pure" B-1 dye by preparative liquid chromatography.

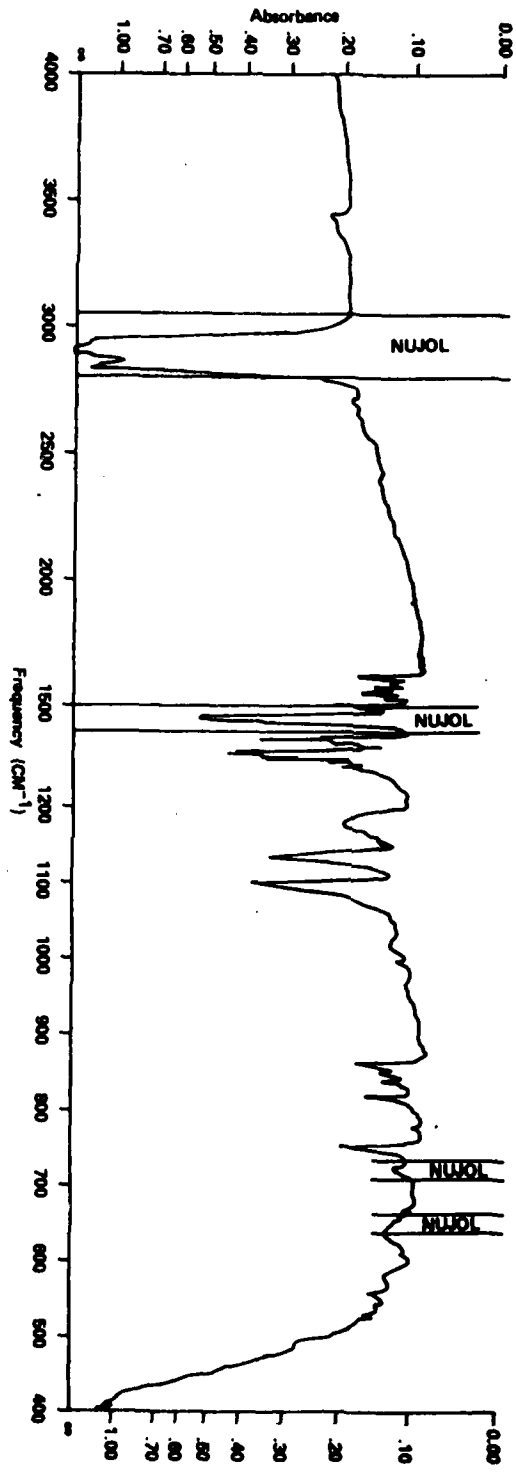

Figure 10. Infra Red Spectrum of B-1 Dye, Sudan Red G, and Mixture of B-1 Dye and Sudan Red G

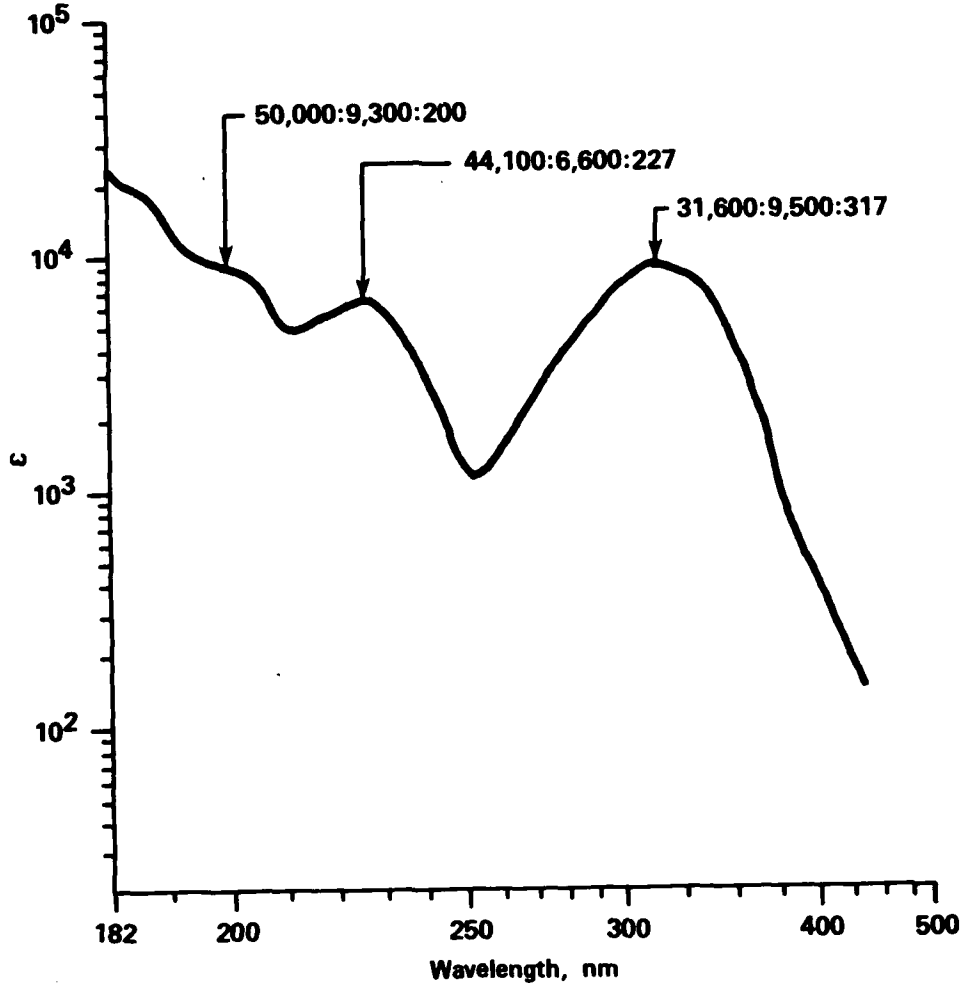

Figure 11. Spectral Characterization of p-Nitrophenol in Water[4]

The technique of dye purity measurement at two wavelengths (absorbance index) demonstrated a method that has considerable utility in distinguishing between dyes of similar structure and color. This technique should be considered in the drafting of the purchase description of B-1 dye.

The present study points out the necessity of preparing high purity B-1 dye for physiochemical measurement of R, the absorbance index value. Other physiochemical values such as melting point, decomposition temperature, etc., should be measured on highly pure B-1 dye.

The application of thin layer chromatography was demonstrated to have utility in separating dye impurities and rapidly giving a qualitative estimate of the types of impurities present. However, it should not be employed as the sole criterion for B-1 dye purity.

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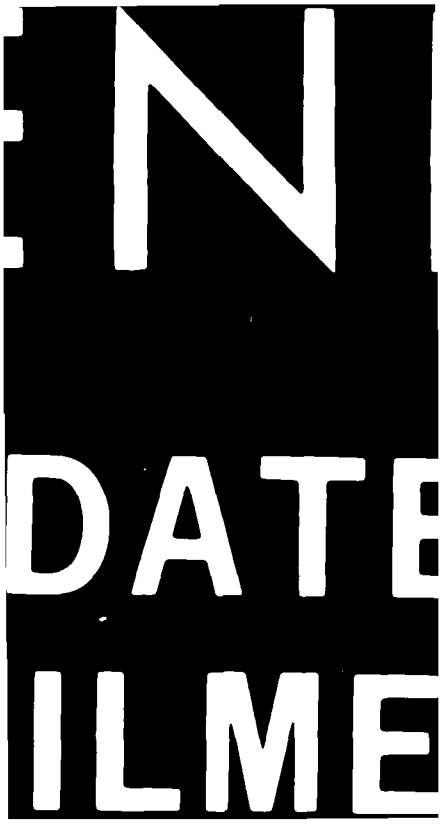
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